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Emesis and Defecations Induced by the 5-Hydroxytryptamine (5-HT_3) Receptor Antagonist Zacopride in the Ferret[1]

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ABSTRACT

Three antiemetic compounds (zacopride, batanopride, granisetron [BRL43694]) were evaluated for the production of gastrointestinal side effects in the conscious ferret after i.v. or p.o. administration. Zacopride evoked multiple emetic and defecatory responses at clinically relevant doses (0.003–0.3 mg/kg) and in a dose-dependent manner. The oral route was the more potent one for eliciting emesis (ED_{50} 0.033 mg/kg). At 0.3 mg/kg p.o., zacopride reliably evoked an 80 to 100% incidence of emesis and a 40 to 80% incidence of defecation. In contrast, batanopride and BRL43694 i.v. evoked a small (10%) incidence of these side effects, but only at 0.1 to 10 mg/kg doses. When given p.o. (0.00003–10 mg/kg), these latter compounds never evoked emesis and significantly reduced ($P < .05$) the incidence of defecation below that of vehicle. Responses to zacopride (0.3 mg/kg p.o.) were challenged by i.p. pretreatment with the 5-hydroxytryptamine receptor agonist 2-methyl serotonin, the 5-hydroxytryptamine receptor antagonist BRL43694, the quaternary atropine derivative glycopyrrolate, the dopamine receptor antagonist domperidone or selective abdominal vagotomies. All compounds and either bilateral or dorsal vagotomy significantly reduced the incidence of emesis, but did not abolish it. Latency to first emesis was delayed by BRL43694, domperidone or dorsal vagotomy. The data suggest that the emetic response to p.o. zacopride is mediated in part by 5-hydroxytryptamine receptors residing on either enteric neurons or vagal afferents. However, the underlying pharmacology of the response may also include activation of cholinergic and dopaminergic pathways.

Several substituted benzamides and 5-hydroxytryptamine (5-HT_3) receptor antagonists are effective antiemetics for radiation- and cytotoxin-induced emesis in many vomiting species (Miner *et al.*, 1987; Bermudez *et al.*, 1988; Dubois *et al.*, 1988; Gylys *et al.*, 1988), including humans (Gralla *et al.*, 1981; Cunningham *et al.*, 1987; Priestman *et al.*, 1988; Kris *et al.*, 1988). As part of their pharmacological action, some of the substituted benzamides also promote gastric motility (Schulze-Delrieu, 1979; Alphin *et al.*, 1986; Cooper *et al.*, 1988). Although the gastric stimulant properties exhibited by some of these compounds can benefit the patient by reinstating normal peristalsis, they may also increase the incidence of diarrhea (Meyer *et al.*, 1984; Kris *et al.*, 1985). The incidence of such unwanted side effects has, in some cases, prompted development of 5-HT_3 receptor antagonist antiemetics with limited gastric stimulant properties (Fake *et al.*, 1987).

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Zacopride (4-amino-N-[1-azabicyclo(2.2.2)oct-3-yl]-5-chloro-2-methyoxybenzamide[E]-2-butenedioate), a substituted benzamide and 5-HT_3 receptor antagonist (Smith *et al.*, 1988b), is a potent antiemetic for radiation- or cytotoxin-induced emesis in dogs, cats, non-human primates, and ferrets (Costall *et al.*, 1987; Cohen *et al.*, 1989; Dubois *et al.*, 1988; Smith *et al.*, 1988a). In the dog, zacopride has been shown to promote gastric motility (Alphin *et al.*, 1986). Recently we observed in the ferret that zacopride, when given i.p. at antiemetic doses, also evoked brief episodes of retching, emesis and defecation during the 20-min period before irradiation (King *et al.*, 1988). Because the emetic phenomenon seemed paradoxical for an antiemetic compound, the studies reported here were initiated to: 1) explore further the dose-response properties of zacopride-induced emesis and defecations; 2) determine whether and to what degree the occurrence of these responses depended on route of administration; and 3) compare the incidence of these side effects to zacopride with their incidence in response to other compounds with somewhat similar antiemetic and pharmacological properties, *i.e.*, granisetron [BRL43694; Endo-N-[9-methyl-9-azabicyclo-(3,3,1)-non-3-yl]-1-methyl-indazole-3-carboxamide; Bermudez *et al.*, 1988] and batanopride (BMY25801; 4-amino-5-chloro-N-[2-(diethylamino)ethyl]2-[1-methyl-2-oxopropoxy]

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); 2-CH_3 5-HT, 2-methyl serotonin; BMY25801, batanopride; BRL43694, granisetron; GI, gastrointestinal; ACh, acetylcholine.

benzamide HCl; Gylys *et al.*, 1988). In addition, emesis to oral zacopride was challenged with anticholinergic, antidopaminergic, or 5-HT_3 receptor-specific ligands, and selective peripheral nerve lesions to determine the underlying receptor specificity and the peripheral anatomical basis for that response.

Methods

Subjects. Experiments were performed on adult male, fitch, castrated and descented ferrets (1–1.5 kg) obtained from Marshall Farms (North Rose, NY). All animals were quarantined upon arrival and screened for evidence of disease prior to release from quarantine. Animals were housed in stainless-steel modified rabbit or cat cages and provided commercial ferret chow and water *ad libitum*. The animal quarters were maintained at 15–21°C, 45 to 55% humidity, and 12-hr light/12-hr dark photocycle.

Catheter implantation. Ferrets with indwelling jugular catheters for i.v. administration of compounds underwent the behavioral adaptation and surgical procedures described by Jackson *et al.* (1988). Briefly, the animals were first adapted to wearing a nylon jacket connected to a stainless-steel cable, which in turn was attached to a brass swivel at the cage top. After habituation to this tether-harness system, each animal received a surgically implanted (under aseptic conditions) catheter in its right jugular vein. The catheter exited the animal between the scapulae and fed through the cable to the swivel where it was capped. The catheter was flushed daily with 0.5 ml of 0.1% heparinized sodium chloride (NaCl; 0.9%). The drug studies began 1 week after the surgical procedure. Animals on tether were individually caged.

Dose dependence and route of administration studies. Eight to 11 individual animals were used to test each dose of each drug administered by any single route. Each animal was weighed weekly and then randomly received, at ≥ 48-hr intervals, a single i.v. or p.o. dose (range of values in parentheses) of one of the following compounds: zacopride (0.00003–0.03 mg/kg i.v.; 0.001–3 mg/kg p.o.), BMY25801 (0.003–10 mg/kg i.v., p.o.), BRL43694 (0.003–10 mg/kg i.v.; 0.003–3 mg/kg p.o.) or 2-methyl-serotonin (2-CH_3 5-HT, 0.003–3 mg/kg i.v., p.o.). Vehicle was 0.9% NaCl for i.v., 5% dextrose and H_2O for p.o. All i.v. injections were given as a bolus in a volume of less than 1 ml; p.o. volume varied between 1 ml and 3 ml; p.o. administration was with a syringe so that the animal was required to swallow the solution.

Pharmacological blockade of emesis and defecation to p.o. zacopride. In order to determine whether and to what degree the emetic response to p.o. zacopride reflected action at 5-HT_3 or other receptors, the following compounds were given as pretreatment (i.p.) 20 min prior to p.o. zacopride (0.3 mg/kg).

1. The 5-HT_3 receptor antagonist BRL43694 (Sanger and Nelson, 1989) was given at a dose equipotent to that of zacopride (0.3 mg/kg). BRL43694 and zacopride show similar inhibition constant values for binding to homogenates of entorhinal cortex (1.98 nM for zacopride, 2.72 nM for BRL43694; Barnes *et al.*, 1988a).

2. The 5-HT_3 receptor agonist 2-CH_3 5-HT was given at doses (1.2 mg/kg or 3 mg/kg) that more closely approximate the difference between the inhibition constant values for it and zacopride (1128 nM for 2-CH_3 5-HT; Barnes *et al.*, 1988a). 2-CH_3 5-HT does not cross the blood-brain barrier.

3. The potent quaternary atropine derivative glycopyrrolate (Robanul®) was given at a dose (0.1 mg/kg) fourfold greater than that which abolishes intestinal contractions in the conscious dog for 20 min (Franko *et al.*, 1962). Glycopyrrolate does not cross the blood-brain barrier and acts primarily on GI function.

4. The D_2-dopamine receptor antagonist, domperidone, was given at a dose (1 mg/kg) that may ameliorate radiation-induced emesis in the dog (Dubois *et al.*, 1984; Cordts *et al.*, 1987). Domperidone does not readily cross the blood brain barrier and has a high affinity for GI tissue (see Brogden *et al.*, 1982).

For these experiments three different groups (A, B and C) of 10 animals were used. Each animal served as its own control. That is, 48 hr prior to challenge with a compound selected from the list just shown, each animal received vehicle (i.p.) as pretreatment, followed 20 min later by zacopride. These data were used to establish both the baseline incidence of emesis/defecation to zacopride and the control values for emetic and defecatory parameters. All animals were retested for their emetic response to p.o. zacopride with vehicle as pretreatment at the conclusion of the experiments.

For group A the emetic response to zacopride was first challenged with BRL43694. After a 48-hr interval, responders to zacopride (vomiting animals) were challenged with glycopyrrolate. Nonresponders (nonvomiting animals) were retested with vehicle pretreatment in order to re-establish that a response to zacopride would occur. After another 48-hr interval, these latter animals were then challenged with glycopyrrolate. For group B the treatment protocols were identical, except that animals were first tested with the lesser dose of 2-CH_3 5-HT, then the greater. For group C only a single dose of domperidone was tested.

Vagotomy studies. Four groups of abdominally vagotomized animals were used in this series of experiments: bilateral ($n = 20$), ventral ($n = 8$), dorsal ($n = 8$) and sham (laparotomy only, $n = 9$). The methods for vagotomy are described in King and Landauer (1990). Each animal was tested for an emetic response to p.o. zacopride (0.3 mg/kg) 48 to 72 hr prevagotomy and 2 to 41 days postvagotomy. The average number of days postvagotomy on which each group was tested was 15 for bilateral, 15 for ventral, 18 for dorsal and 36 for sham.

Data collection and statistical analysis. Individual animals were observed for 30 min following: 1) i.v. and p.o. drug administration to evaluate dose-response effects; and 2) p.o. zacopride administration in control conditions, *e.g.*, with vehicle pretreatment or prevagotomy. The observation period was 1 hr after challenge of response to p.o. zacopride with receptor ligands or vagotomy. The observer recorded the frequency of, and latency to, all expulsions, retches and defecations. Episodes of emesis and retching separated by ≥ 4 min were considered single events. The following parameters were analyzed from all recorded data: 1) latency to first emesis or retch; 2) number of emetic or retching *episodes*; 3) number of expulsions and/or retches; and 4) latency to and number of defecations (including attempts to defecate). Parameters 1 to 3 can be individually altered by vagotomy and/or 5-HT_3 receptor antagonist treatment (Andrews and Hawthorn, 1987; Hawthorn *et al.*, 1988; Andrews, *et al.*, 1990). All parameters were tabulated as mean ± S.E. for vomiting and retching animals in each group. For graphic representation in the dose-response curves, vomiting and retching-only animals were pooled. Data obtained from the dose-response curves were tested for statistical significance by chi-square analysis of proportions using a contingency table. The ED_{50} value for emesis to oral zacopride was determined by log-probit analysis. Data obtained from the pharmacologic pretreatments and nerve lesions were compared with the test for significance of difference between two proportions and the Student's *t* test (Bruning and Kintz, 1977). Statistical significance was assumed when $P < .05$.

Drugs used. All drugs were prepared weekly (except 2-CH_3 5-HT and domperidone, which were prepared daily) and refrigerated. For p.o. administration, the compounds were dissolved in a 5% dextrose and water solution, for i.v. or i.p. administration, a 0.9% NaCl solution. Domperidone was prepared and injected as a suspension made from a 2% carboxymethyl cellulose vehicle in NaCl. 2-CH_3 5-HT was obtained from Research Biochem., Inc. (Natick, MA), both zacopride and glycopyrrolate are compounds from the research of A. H. Robins (Richmond, VA); BMY25801, Bristol-Myers (Wallingford, CT); and BRL43694, Beecham Pharmaceuticals (Harlow, Essex, UK). Domperidone was a gift from Janssen Pharmaceutica, Inc. (Piscataway, NJ). Doses of BRL43694 refer to the base; all others to the salt.

Results

Emesis and defecations to antiemetic compounds. Figure 1 illustrates that, of the three antiemetics tested for an emetic response by either the i.v. or p.o. administration route, only zacopride produced any significant incidence of emesis.

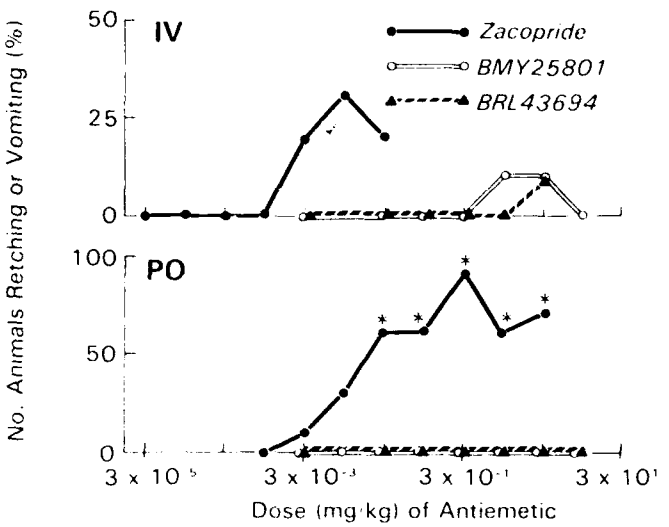

Fig. 1. Dose-dependent incidence of emetic or retching responses to zacopride contrasted with BRL43694 and BMY25801 as a function of administration route. Ferrets observed for 30-min period after drug administration. Top: response to i.v. (bolus) administration. All compounds were dissolved in NaCl. Each data point for BMY25801 and BRL43694 represents tests in eight to 10 individual animals; zacopride, 10 to 11. No emetic/retching responses to vehicle were observed (n = 30). Bottom: response to p.o. administration. All compounds were dissolved in 5% dextrose/H_2O. Each point represents tests in 10 to 14 animals. Statistical significance (*$P < .05$) for individual data points was seen at all doses of zacopride greater than 0.030 mg/kg. For the entire dose-response curve, 42% of those animals tested vomited after zacopride ($P < .05$), compared with none for BMY25801 or BRL43694. No emetic/retching responses to vehicle were observed (n = 30).

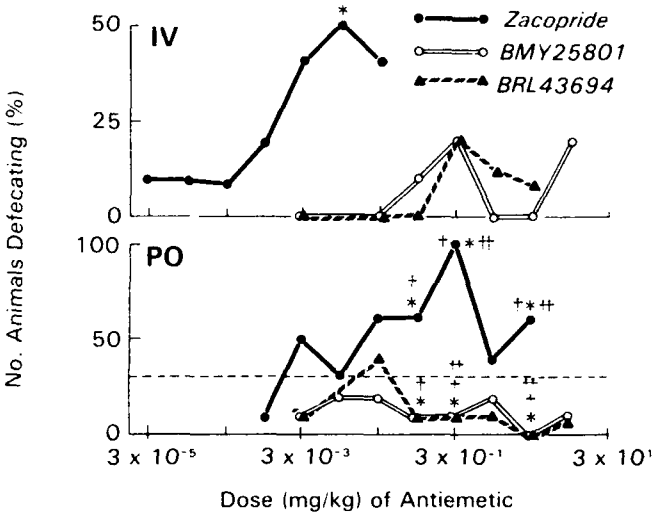

Fig. 2. Dose-dependent incidence of defecatory responses to zacopride contrasted with BRL43694 and BMY25801 as a function of administration route. Ferrets observed for 30-min period after drug administration. Top: response to i.v. (bolus) administration. All compounds were dissolved in NaCl. Each data point for BMY25801 and BRL43694 represents tests in eight to 10 individual animals; zacopride, 10 to 11. No defecatory responses to vehicle were seen (n = 30). Bottom: response to p.o. administration. All compounds were dissolved in 5% dextrose/water. Each data point represents tests in 10 to 14 animals. Significant individual differences were observed among compounds (*, †) and vehicle (††) at doses equal to or greater than 0.1 mg/kg ($P < .05$). For all doses that ranged from 0.003 to 3 mg/kg, defecation to zacopride was 62%; BMY25801, 12%; BRL43694, 13%. This incidence of defecation to zacopride differed significantly ($P < .05$) from that for BMY25801, BRL43694 and vehicle (dashed line, n = 10/30). The incidence of defecation to both BMY25801 and BRL43694 was significantly ($P < .05$) less than that of vehicle.

When given i.v. at doses ranging between 0.003 and 0.3 mg/kg, zacopride evoked a 20 to 30% (n = 10/dose) incidence of emesis or retching at each dose. In contrast, neither BMY25801 nor BRL43694 given i.v. evoked emesis at doses less than 1 mg/kg. BMY25801 elicited a 10% incidence of emesis at 1 to 3 mg/kg (n = 10/dose) and BRL43694 evoked a 9% incidence of emesis at 3 mg/kg (n = 1/11). The total incidence of emesis to all i.v. doses of zacopride (9.9%) did not significantly differ from the total incidence for all i.v. doses of BMY25801 (3%) or BRL43694 (2%). When given p.o., the emetic responses to zacopride were more pronounced than when given i.v., whereas BMY25801 and BRL43694 failed to elicit emesis at any dose tested. 2-CH_3 5-HT (data not shown) produced only a single emetic episode when given i.v. (3 mg/kg; n = 1/9), but never at lower i.v. doses (n = 38, 9–10/dose) or any p.o. dose (n = 40, 9–10/dose). Although most emetic responses were prompt, the range of emetic latencies varied from 2 to 25 min, regardless of administration route or compound. No emesis was observed to i.v. or p.o. vehicle. The emetic responses to both i.v. and p.o. zacopride also appeared dose dependent. The ED_{50} value for emesis to p.o. zacopride was calculated as 0.033 mg/kg (95% confidence limits = 0.015, 0.077 mg/kg).

Figure 2 shows that zacopride also evoked a greater incidence of a defecatory response than did BMY25801 or BRL43694, regardless of administration route. When given i.v. at doses ranging between 0.00003 and 0.03 mg/kg, zacopride produced a 9 to 50% incidence of defecation that appeared dose dependent (n = 10–11/dose). In contrast, a low incidence of defecations occurred in response to BMY25801 (n = 4/40) and BRL43694 (n = 3/50), but only at doses greater than or equal to 0.1 mg/kg. A defecatory response was never seen after vehicle. Like the emetic response, the defecatory response to p.o. zacopride was more pronounced than when given i.v. and it too appeared to be dose dependent. In p.o. doses that ranged from 0.003 to 3 mg/kg, the overall incidence of defecation was 62% for zacopride, 12% for BMY25801 and 13% for BRL43694. The 62% incidence of defecation to zacopride was significantly greater ($P < .05$) than for BMY25801, BRL43694 or vehicle (n = 10/30). In contrast, the incidence of defecation to either BMY25801 or BRL43694 was significantly lower ($P < .05$) than for vehicle. Animals (n = 6/33) also defecated after all p.o. doses of 2-CH_3 5-HT (data not shown), but the incidence did not differ from that for vehicle.

Analysis of the dose-response data for zacopride showed that both the emetic and defecatory responses were likely to occur with similar frequency to an identical dose of the drug. That is, the dose-response curves for the two side effects appeared identical for i.v. zacopride ($r = 0.9754$, $P < .05$) and very similar for p.o. zacopride ($r = 0.7094$, $P < .10$). In addition, the responses were likely to occur in the same animal. No such correlation between the two side effects was observed for either BRL43694 or BMY25801. The latency to emesis after p.o. zacopride (0.003–0.3 mg/kg) was inversely correlated with the dose of zacopride ($r = -0.8119$, $P < .05$) whereas the latency to defecation was not ($r = -0.1949$); p.o. zacopride (>0.003 mg/kg) also produced multiple episodes of both emesis/retching and defecation. Multiple episodes of emesis or defecation never occurred in response to BMY25801, BRL43694, 2-CH_3 5-HT or vehicle.

Behavioral responses other than emesis and defecation were

also observed after i.v. zacopride. At a threshold dose of 0.001 mg/kg all doses of zacopride produced a brief behavioral arousal that was characterized by standing, then circling the cage. The arousal period was followed by 20 to 30 sec of an increased depth and rate of respiration (panting), then 5 to 20 min of apparent listlessness or sedation from which the animal could be aroused. Because it was unclear as to whether the apparent sedation was life-threatening, zacopride was not tested at greater i.v. doses. Salivation was also occasionally observed, but none of these behavioral responses was explored in detail. After 2-CH_3 5-HT was given i.v., brief episodes of arousal (0.03–3 mg/kg, n = 25/39), panting (0.03–3 mg/kg, n = 14/39) and listlessness (0.1–3 mg/kg, n = 15/29) were also observed. Panting was observed after i.v. BRL43694 only at the highest dose tested (3 mg/kg, n = 1/11). No similar, or other, behavioral effects were observed in response to i.v. BMY25801 or vehicle. These responses were never observed with any compound given p.o.

Pharmacological antagonism of emesis and defecations to p.o. zacopride by 5-HT_3 receptor ligands, 2-CH_3 5-HT and BRL43694. The lower dose (1.2 mg/kg) of 2-CH_3 5-HT reduced the incidence of zacopride-induced emesis from 90 to 50% but that reduction was not statistically significant (table 1). This dose of 2-CH_3 5-HT also failed to affect the latency to emesis, the incidence of defecations or their latency to onset. However, as seen in table 1, both the larger dose of 2-CH_3 5-HT and BRL43694 significantly reduced the incidence of emesis to oral zacopride, but did not abolish it. Vomiting animals were also protected by these compounds, as evidenced by the significant increased latency to first emesis by BRL43694. Both compounds also significantly reduced the number of retches. The frequency of retches returned to control values during the second 30-min observation period after BRL43694 and 1.2 mg/kg 2-CH_3 5-HT (data not shown). The number of expulsions was not altered by either compound (data not shown). During the second 30-min observation period after BRL43694 pretreatment an additional animal vomited. Although BRL43694 did not affect the defecatory response to zacopride, 2-CH_3 5-HT at 3 mg/kg totally abolished it.

Pharmacological antagonism of emesis and defecations to p.o. zacopride by the muscarinic or dopamine receptor antagonists, glycopyrrolate or domperidone. As seen in table 2, both compounds significantly reduced the incidence of emesis to oral zacopride, but did not abolish it. During the second 30-min observation period after glycopyrrolate pretreatment, an additional animal vomited. The latency to the first emetic episode was significantly increased by domperidone pretreatment. Table 2 also shows that both compounds completely abolished the defecatory response to zacopride for the first 30-min observation period. Two glycopyrrolate-treated animals and one domperidone-treated animal defecated during the second 30-min observation period.

Vagotomy *vs.* emesis and defecation to oral zacopride. Table 3 shows that both bilateral and dorsal abdominal vagotomy significantly ($P < .05$) reduced the incidence of emesis to zacopride. Bilateral vagotomy also: 1) reduced (but not significantly) the number of retches; and 2) abolished expulsions to zacopride in the two responding animals. Dorsal vagotomy significantly increased the latency to first emesis ($P < .05$); ventral abdominal vagotomy had no effect. Of those seven animals that vomited to zacopride treatment after ventral vagotomy, five had multiple episodes. Only in two animals (one ventral, one sham) did an emetic episode occur during the second 30-min observation period postvagotomy.

Sham vagotomy had a marked effect on the emetic response to oral zacopride. After this procedure significantly more animals ($P < .05$) vomited after zacopride treatment. In addition, the number of both expulsions and retches significantly increased in the responding animals ($P < .05$).

The latency of the defecatory response to p.o. zacopride was significantly increased by dorsal vagotomy ($P < .05$). Another dorsally vagotomized animal defecated during the second 30-min observation period. Sham vagotomy caused a significant reduction in the incidence of defecations ($P < .05$). For those animals (n = 8) that showed multiple defecations to zacopride prior to vagotomy, this incidence was reduced to 25% after bilateral or dorsal vagotomy (data not shown).

Discussion

Dose-response curves for emesis and defecations to zacopride, BMY25801 and BRL43694. The principal and most significant finding of this study is that dose-dependent *emetic* responses were evoked in the ferret by i.v. or p.o. administration of the *antiemetic* zacopride, a compound that is being evaluated for clinical use. Zacopride also evoked dose-dependent defecatory responses and other behavioral changes. These side effects to zacopride occurred at clinically relevant,

TABLE 1

Suppression of emetic or defecatory responses to zacopride (0.3 mg/kg p.o.) by pretreatment with the 5-HT_3 antagonist BRL43694 or the 5-HT_3 agonist 2-CH_3 5-HT

Values are means ± S.E.

Parameter	Pretreatment[a]					
	Vehicle	BRL43694	Vehicle	2-CH_3 5-HT_1	Vehicle	2-CH_3 5-HT_2
No. vomiting or retching/tested	9/10	3/10*	9/10	5/10	9/10	1/10*
Latency to 1st episode (min)	13.3 ± 3.0	26.1 ± 2.6*	9.4 ± 2.8	15.2 ± 4.0	10.7 ± 1.9	[6.0][b]
No. retches	17.1 ± 5.1	5.7 ± 0.7*	14.9 ± 2.4	3.0 ± 2.5*	11.6 ± 2.0	[6.0]
No. defecating/tested	6/10	7/10	4/10	4/10	5/10	0/10*
Latency to defecation (min)	9.4 ± 3.1	18.1 ± 4.1	6.0 ± 2.2	13.8 ± 4.3	6.8 ± 3.1	—

[a] Vehicle (NaCl) or drug (BRL43694, 0.3 mg/kg; 2-CH_3 5-HT_1, 1.2 mg/kg; 2-CH_3 5-HT_2, 3 mg/kg) given i.p. 20 min before zacopride administration. All data were recorded during the 1st 30-min interval after zacopride administration.

[b] Numbers in brackets represent n = 1.

* Statistical significance ($P < .05$) between vehicle and drug pretreatment by the test for significance of difference between two proportions or the Student's t test for paired data.

TABLE 2

Suppression of emetic or defecatory responses to zacopride (0.3 mg/kg p.o.) by pretreatment with a muscarinic (glycopyrrolate) or dopamine (domperidone) receptor antagonist

Values are means ± S.E.

Parameter	Pretreatment[a]			
	Vehicle	Glycopyrrolate	Vehicle	Domperidone
No. vomiting or retching/tested	9/10	4/10*	10/10	5/10*
Latency to 1st episode (min)	14.8 ± 3.0	20.0 ± 3.4	6.2 ± 1.7	14.4 ± 4.0*
No. retches	10.9 ± 2.5	15.0 ± 4.6	12.4 ± 1.9	8.6 ± 1.6
No. defecating/tested	5/10	0/10*	4/10	0/10*
Latency to defecation (min)	8.0 ± 2.8	57.5 ± 0.5[b]*	7.5 ± 1.9	—

[a] Vehicle (NaCl) or drug (glycopyrrolate, 0.1 mg/kg; domperidone, 1 mg/kg) given i.p. 20 min before zacopride administration. All data (except [b]) were recorded during the 1st 30-min interval after zacopride administration.

[b] Recorded during the 2nd 30-min interval after zacopride administration.

* Statistical significance ($P < .05$) between vehicle and drug pretreatment by the test for significance of difference between two proportions or the Student's t test for paired data.

antiemetic doses (Costall *et al.*, 1987; King and Landauer, 1990). The actions of zacopride contrasted significantly with the actions of BMY25801 and BRL43694, antiemetics with profiles somewhat similar to those of zacopride in classic models for testing 5-HT_3 receptor characteristics of compounds. BMY25801 and BRL43694 evoked only isolated emetic responses when given i.v. and never when given p.o. Defecatory responses to these latter compounds were not dose-dependent when given i.v. and were significantly less than vehicle when given p.o.

Emesis and defecation in response to zacopride were previously observed in ferrets after i.p. administration (King *et al.*, 1988; King and Landauer, 1990). Emesis to p.o. zacopride in ferrets has recently been confirmed by Sancilio *et al.* (1990) and their ED_{50} value (0.03 mg/kg; A. H. Robins, personal communication) for that emetic response is similar to that reported here (0.033 mg/kg). In contrast, Costall *et al.* (1987) reported no side effects to i.v. zacopride in ferrets, but the animals were anesthetized for its administration and then allowed 15 to 20 min to recover. The sedation associated with recovery from anesthesia in that study may have prevented or masked the side effects reported here. Others have reported no side effects in response to doses of zacopride ranging from 0.00003 to 0.5 mg/kg (i.v., p.o. or i.p.) in cats (Smith *et al.*, 1988a), dogs (Alphin *et al.*, 1986; Cohen *et al.*, 1989; Smith *et al.*, 1988b), nonhuman primates (Dubois *et al.*, 1988; Sancilio, personal communication) and humans (Sancilio, personal communication). The lack of observed side effects in these studies probably reflects differences among species and routes of administration.

The isolated instances of emesis and defecation to i.v. BMY25801 and BRL43694 have also not been previously observed (Gylys *et al.*, 1988; Bermudez *et al.*, 1988). However, the doses of BMY25801 and BRL43694 evaluated in this study and that evoked these responses exceeds the doses used by those authors. In addition, emesis and defecations as side effects could have been overlooked by those authors. These events could have been masked by emesis/defecations that occur normally in response to cytotoxins and radiation. For example, BMY25801 was not fully protective for emesis against these latter stimuli (Gylys *et al.*, 1988; King and Landauer, 1990). In humans also, emesis has not been reported in response to either BMY25801 or BRL43694 when given i.v. (Zussman *et al.*, 1988; Carmichael *et al.*, 1988; Cassidy *et al.*, 1988; Plezia *et al.*, 1988; Smaldone *et al.*, 1988). Although no GI side effects were reported for BRL43694, 38% of patients receiving BMY25801 reported loose stools (Plezia *et al.*, 1988). This latter pattern of clinically observed GI side effects was found in the present study in ferrets.

It is especially noteworthy that both BMY25801 and BRL43694, when given p.o., significantly reduced the incidence of defecations when compared with vehicle. Such data suggest that, when given orally, these compounds could be useful for counteracting lower GI motility disorders.

Emetic response to oral zacopride. The selective action of dorsal vagotomy to reduce the incidence of emesis to oral zacopride suggests that the predominant afferent pathway for initiation of that emetic response is the dorsal vagus nerve. In ferrets, the dorsal vagus primarily innervates the dorsal aspects of the stomach and the coeliac ganglion (MacKay and Andrews, 1983). Andrews *et al.* (1990) have reported that the emetic latency to certain compounds given p.o. (*e.g.*, copper sulfate) to the ferret varies inversely with the time of recovery after vagotomy. It is thus unlikely that the increased latency to emesis after dorsal vagotomy is confounded by the time of testing with zacopride.

TABLE 3

Effect of vagotomy on emetic or defecatory responses to zacopride (0.3 mg/kg p.o.)

Values are means ± S.E.

Parameter[a]	Sham		Vagotomy					
			Bilateral		Unilateral			
					Dorsal		Ventral	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
No. vomiting or retching/tested	5/9	9/9*	16/20	2/20*	8/8	2/8*	6/8	7/8
Latency to 1st episode (min)	13.8 ± 3.4	8.6 ± 1.6	11.9 ± 1.9	13.5 ± 5.5	12.2 ± 2.2	23.0* ± 2.0	11.2 ± 1.7	12.4 ± 1.9
No. expulsions	1.2 ± 0.4	2.3* ± 0.3	1.4 ± 0.2	0	1.7 ± 0.2	1.0 ± 0.0	1.4 ± 0.3	1.7 ± 0.2
No. retches	6.4 ± 2.4	14.6* ± 2.4	10.3 ± 4.8	3.0 ± 2.0	—	—	—	—
No. defecating/tested	4/9	0/9*	13/20	11/20	5/8	4/8	0/8	1/8
Latency to defecation (min)	13.0 ± 5.1	—	13.1 ± 2.6	9.2 ± 1.1	8.6 ± 2.0	21.3* ± 4.7	—	22.0

[a] All data were recorded for 30 min after zacopride administration; — indicates that data were not recorded.

* Statistical significance ($P < .05$) between pre- and postvagotomy values by the test for significance of difference between two proportions or the Student's t test for paired data.

The antiemetic action of glycopyrrolate implies that zacopride may facilitate ACh release from myenteric neurons, which in turn may play some role in the emesis to zacopride. Zacopride enhances the ACh-mediated muscle-twitch in guinea-pig ileum (Craig and Clarke, 1989) but does not bind at muscarinic ACh receptors (Barnes *et al.*, 1988a). Likewise, the antiemetic action of domperidone also suggests a role for dopamine in mediating this emetic response. Although the precise role for a dopaminergic mechanism in zacopride-induced emesis is unclear from these data, other evidence suggests that central 5-HT_3 and dopamine receptors interact (see Tricklebank, 1989). Costall *et al.* (1989) recently reported that the 5-HT_3 receptor antagonist ICS 205-930 can partially prevent apomorphine-induced emesis in the ferret.

The data also strongly suggest that the emesis to p.o. zacopride results from an action at the 5-HT_3 receptor. This was demonstrated by the reduced emesis seen after pretreatment with either the 5-HT_3 receptor antagonist BRL43694 or agonist 2-CH_3 5-HT. Although emesis was not completely abolished by either compound, those animals still vomiting after BRL43694 exhibited both a significantly: 1) increased latency to the first emetic episode; and 2) reduced number of retches. Likewise, the number of retches was significantly reduced by the lower dose of 2-CH_3 5-HT. The action by the 5-HT_3 receptor ligands to ameliorate retching but not vomiting suggests that subtle pharmacological differences exist between the underlying mechanisms for the two events. Such a differential effect on vomiting *vs.* retching has been observed with zacopride and BRL43694 when used as antiemetics for cytotoxin- and radiation-induced emesis (Andrews and Hawthorn, 1987; Dubois *et al.*, 1988; Hawthorn *et al.*, 1988). These authors, however, found a greater percentage decrease in emetic rather than retching events.

If one accepts that the emetic response to p.o. zacopride is mediated predominantly by 5-HT_3 receptor action, the reduced emesis in response to dorsal vagotomy implies that these receptors are distributed primarily along the dorsal wall of the stomach, possibly within the myenteric plexus or along vagal afferents. In support of this notion, Bingham (1987) has shown that close intra-arterial injections of 5-HT and 5-hydroxytryptophan in μg/kg doses will evoke an (atropine-sensitive) antral contraction in the stomach of the anesthetized ferret. However, a separate (peripheral or central) pathway must be involved in the emetic response to oral zacopride since vagotomy does not completely abolish the emesis.

Several alternative explanations exist that can account for the partial abolition of zacopride-induced emesis by the 5-HT_3 receptor ligands. For example, because 5-HT_3 receptors are found throughout the peripheral nervous system (Bradley *et al.*, 1986), BRL43694 and 2-CH_3 5-HT could act at other sites along the emetic pathway to prevent the emesis. Their distribution or duration of action could also vary across individual animals. It seems unlikely that the specific challenging doses chosen for these compounds were inadequate. Of the three pretreatments, only BRL43694 failed to abolish the defecatory response to zacopride.

The enhanced emetic responses to zacopride following sham vagotomy were unexpected. Although the reason for this response is unknown, it could reflect an increased level of GI 5-HT production in the response to anesthesia and laparotomy. According to Gomes *et al.* (1985), this may last for up to 5 weeks postvagotomy.

Defecatory response to oral zacopride. In contrast to the emetic response to oral zacopride, the incidence of a defecatory response was totally abolished by glycopyrrolate, domperidone and the higher dose of 2-CH_3 5-HT. Its latency to onset was also increased by dorsal vagotomy. Because oral zacopride enhances gastric emptying (Dubois *et al.*, 1988), that preparation may have passed into the intestine and activated the more proximal myenteric neurons to stimulate peristalsis. This could explain the rapidity of the defecatory response and the fact that dorsal vagotomy significantly increased the latency of this response. The vagus does contain two motor pathways that are reflexly excited by vagal afferent input, one of which is cholinergic (Collman *et al.*, 1984a,b). However, such a mode of action for zacopride can only be speculated because the distribution of vagal fibers in the ferret has not been traced anatomically beyond the coeliac ganglion (MacKay and Andrews, 1983).

Other side effects to zacopride administration. It was also observed that clinically relevant doses of i.v. zacopride, but not BMY25801 or BRL43694, produced sedation or listlessness, which was preceded by a central arousal and labored respiration. We have previously reported sedative-like response to i.p. zacopride, expressed as a depression of vertical exploratory behavior (King and Landauer, 1990). In contrast, Costall *et al.* (1987) reported that their animals remained alert and active during the recording period. Such differences could be attributed to varied observation methods (see King and Landauer, 1990).

The arousal and labored respirations followed by listlessness induced by low doses of i.v. zacopride were mimicked only by similar doses of i.v. 2-CH_3 5-HT. Although the arousal and listlessness from zacopride may result from central action, 2-CH_3 5-HT does not cross the blood-brain barrier. It is thus likely that all of these side effects were in response to action on peripheral 5-HT_3 receptors by these compounds. Activation of 5-HT_3 receptors in the epicardium, juxtapulmonary capillary bed and carotid body produce numerous reflexive autonomic responses. These peripheral responses, which include bradycardia, hypo- and hypertension, rapid shallow breathing and inhibition of somatic muscles (for review, see McQueen and Mir, 1989), could account for the observed behaviors.

General significance of results. The most compelling result from the pharmacological studies was that emesis to oral zacopride was not totally abolished by any single compound. In contrast, the defecatory response was completely abolished by glycopyrrolate, domperidone and 2-CH_3 5-HT. Based on these data, the most likely explanation for these results is that zacopride acts in the GI tract in a manner analogous to that of metoclopramide, which stimulates motility by facilitating ACh release from myenteric neurons (Schulze-Delrieu, 1979; Sanger, 1985, 1987). That zacopride enhances ACh-mediated contractions in the guinea-pig ileum (Craig and Clarke, 1989), an action that is mimicked by 5-HT (Sanger, 1985, 1987), supports this hypothesis. As suggested by the action of domperidone, however, zacopride may also facilitate dopamine release from the myenteric plexus, but this has yet to be confirmed *in vitro*. Zacopride antagonized 2-CH_3 5-HT-induced contractions of guinea-pig ileum (Cohen *et al.*, 1989), but the transmitter mediating these contractions was not defined. It is also possible that the antidefecatory action of these receptor antagonists reflects a nonspecific action by zacopride on GI motility. The incidences of a defecatory response to NaCl plus zacopride

during one series of experiments (vehicle: tables 1 and 2) were not significantly greater than that to p.o. vehicle alone in another (fig. 2 *lower, dashed line*; $n = 10/30$).

Undoubtedly, the mechanism by which zacopride evokes an emetic and/or defecatory response varies with the route of administration. The emetic responses to i.v. zacopride may result from action at the chemoreceptor trigger zone in the area postrema (Borison and Wang, 1953). 5-HT_3 receptor binding has been shown in the ferret area postrema (Barnes *et al.*, 1988b) and emesis to i.v. neurotransmitter receptor agonists is abolished by area postrema lesion (Carpenter *et al.*, 1984). A similar action at the chemoreceptor trigger zone might explain the emetic responses to i.v. BMY25801 and BRL43694, although the greater doses necessary to evoke emesis would suggest some other, non-5-HT_3 selective action. Alternatively, emesis to i.v. zacopride could be in response to activation of epicardial 5-HT_3 receptors (see McQueen and Mir, 1989). The mechanism for evoking a defecatory response after i.v. administration is unknown.

It can only be speculated as to what attribute zacopride has that would explain its ability to evoke emetic or defecatory responses and thus distinguish it so markedly from BMY25801 and BRL43694. Both zacopride (Smith *et al.*, 1988b) and BRL43694 (Sanger and Nelson, 1989) are potent 5-HT_3 receptor antagonists, but the former stimulates gastric motility (Alphin *et al.*, 1986) and the latter does not (Sanger and Nelson, 1989). BMY25801 has not been tested for 5-HT_3 receptor affinity (Monkovic *et al.*, 1988). All three compounds attenuate the Bezold-Jarisch reflex in a dose-response fashion, although BMY25801 only at greater doses (Gylys *et al.*, 1988). The complete pharmacological profile exists only for BRL43694.

Zacopride could act as either a partial agonist or mixed agonist-antagonist at the 5-HT_3 receptor (see Craig and Clarke, 1989). Such agonist action would explain the finding that i.v. zacopride and 2-CH_3 5-HT evoke similar physiological responses and that zacopride can be both emetic and antiemetic. However, agonist action by zacopride would not explain an emetic response to zacopride but not 2-CH_3 5-HT. Alternatively, zacopride is a racemate and the isomers show different effects on gastric emptying (Wade *et al.*, 1989). Thus the emetic or antiemetic and other properties of zacopride may be related to a specific stereoisomer.

In summary, the 5-HT_3 receptor antagonist and antiemetic zacopride has also been shown to demonstrate emetogenic properties at antiemetic doses. This was not seen in response to two other somewhat similar compounds, BMY25801 and BRL43694. The emetic response to zacopride was most pronounced when it was given orally and that emetic response was attenuated, but not abolished, by prior administration of various receptor ligands or dorsal vagotomy. That zacopride shows both emetic and antiemetic properties at similar doses is a paradox that has yet to be resolved.

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